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THE RELIABILITY OF THE SACHS-GEORGI TEST FOR SYPHILIS

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As early as 1907 Michaelis,¹ Levaditi and Yamanouchi² and several other investigators expressed the opinion that a precipitate was formed in a positive Wassermann test. Jacobsthal³ was able to show, with the aid of the ultramicroscope, that such a precipitate could actually be observed when a mixture of Wassermann antigen and syphilitic serum were incubated for half an hour at 37 C. Many workers in the years following tried to devise practical methods of visible precipitation which would be specific and reliable in the diagnosis of syphilis. Lang,⁴ in 1912, published the colloidal gold test which has proved to be a helpful method in the diagnosis of cerebrospinal syphilis. The method of Sachs and Georgi⁵ was published in 1918, and soon after this these workers were able to summarize results of 12,124 tests made according to their method in their own laboratory and in those of other workers following their directions. In this series there was an agreement of 92.44% with parallel Wassermann reactions. Because of the simplicity of this test, numerous workers have investigated its reliability. The agreement in the results obtained with parallel Wassermann tests, has been so encouraging that the Sachs-Georgi test is receiving more and more serious consideration as a substitute for the Wassermann reaction in situations in which it is difficult to obtain proper conditions for the successful handling of the more complicated procedure.

Parker and Haigh⁶ of this laboratory have published the results of 520 Sachs-Georgi tests made parallel with the Wassermann test, showing an agreement of 93.07%. The methods were essentially those originally devised by Sachs and Georgi. Since then, D'Aunoy⁷ reported

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¹ Berl. klin. Wehnschr., 1907, 44, p. 1477.

² Compt. rend. Soc. d. biol., 1907, 59, p. 740.

³ München. med. Wehnschr., 1905, 56, p. 2607.

⁴ Berl. klin. Wehnschr., 1912, 49, p. 897.

⁵ Med. Klin., 1918, 14, p. 805.

⁶ Arch. Dermat. & Syph., 1921, 4, p. 67.

⁷ Jour. Med. Research, 1921, 42, p. 339.

an agreement of 98.07% in 2,150 hospitalized cases. Taniguchi,⁸ in England, reported an agreement of 91.2% in 296 cases. In general, the greatest divergence of results occurred in weak positive and doubtful reactions. Levinson and Peterson⁹ reported agreement of 78% on serums and spinal fluids in 100 cases of tabes, paresis, cerebrospinal syphilis, etc. In tests carried out on many serums from syphilitic patients in different stages of the disease, Stern¹⁰ found that the Sachs-Georgi test agreed with the Wassermann reaction in 88% of the cases. In spinal fluids there was agreement of 86% in the 2 tests. It was observed in this series that in primary and secondary syphilis the Wassermann test was stronger than the Sachs-Georgi, whereas in latent or treated cases of syphilis the reverse was true. Tannenberg¹¹ cited the collected reports of Pesch from 30 investigators who had compared the Wassermann and Sachs-Georgi tests in 31,000 cases in which there was an agreement of 86.9% of the cases tested. Summarizing from the results obtained by 44 investigators, involving 56,586 cases, it appears that the Sachs-Georgi test agree with the Wassermann test in 88.7% of the tests.

THE REACTION

To obtain the results here reported we have used the technic described by Parker and Haigh, introducing a few minor modifications which expedited setting up the tests and assured the production of an antigen of the proper sensitiveness with somewhat more regularity.

Antigen Extract.—The antigen was prepared from fresh beef heart, trimmed free of fat and connective tissue, and ground fine by passing several times through a meat chopper equipped with the "peanut butter" grinding head. The chopped heart was then mixed with an equal bulk of clean sand, which had previously been thoroughly washed and ignited to remove salts and organic matter, and was ground in a mortar until the mass had the consistency of stiff putty. It was then placed in a ground-glass stoppered bottle containing a few glass beads. Five volumes of absolute alcohol were added and the extraction was effected by shaking mechanically for 5 hours, and then allowing to stand at room temperature for 24 hours. The mass was then filtered through filter paper and placed in the icebox for 48 hours to allow the precipitation of phosphates. These precipitates were removed by filtration

⁸ Brit. Jour. Exper. Path., 1921, 2, p. 41.

⁹ Jour. Nerv. & Ment. Dis., 1921, 54, p. 413.

¹⁰ Ztschr. f. Immunitätsf. u. exper. Therap., 1921, 32, p. 167.

¹¹ Ibid., p. 381.

while the mass was still cold, and the clear antigen extract was placed in tightly stoppered bottles and kept at room temperature.

Titration of the Antigen.—The most important part of the technic in this test is the titration of the antigen, a procedure in which we followed with almost no modification the details as described by Sachs and Georgi and somewhat modified by Parker and Haigh. For specific directions, therefore, we may refer the readers to the last paper. In principle, the procedure consisted in making 3 mixtures of the crude antigen in which 1, 2 and 3 volumes of absolute alcohol were added to 1 volume of the antigen, respectively. Each of these antigen alcohol dilutions, then, was divided into four parts, and to these was added a 1% solution of cholesterol in absolute alcohol sufficient in amount to bring the several fractions to 0.03, 0.045, 0.06 and 0.075% of the cholesterol solution. These cholesterolized antigen dilutions were then diluted with 5 volumes of salt solution. We thus obtained 12 different antigen mixtures, or 3 sets of 4 each, each set containing 1 tube each of equivalent cholesterol concentrations on a varying antigen-alcohol mixture basis. Titration against known positive and negative serums were then made with these in the usual manner and after the first few titrations, controls made with the same serums and an antigen used successfully in previous tests. In general, our optimum antigen dilution was one in which 1 volume of antigen extract had been mixed with 5 volumes of salt solution as described. The actual steps in the method of adding the antigen to the tests is described in detail further on. Two antigens of about equal sensitiveness were used for all the tests made.

Considerable difficulty was experienced in producing good antigens by extracting with 95% alcohol. Absolute alcohol was tried and satisfactory antigens were produced which were reliable. It was found, too, that a 1% solution of cholesterol would remain in solution at room temperature in absolute alcohol, whereas the cholesterol would crystallize out of the 1% solution in 95% alcohol, which necessitated gentle heating to produce complete solution before using each time.

Patient's Serum.—The patient's serum should be clear, fresh and inactivated by heating for one half hour at 56 C. The serum should be allowed to stand 3 or 4 hours after inactivation before the tests are made. Serums used too soon after inactivation were found by Munster¹² to give nonspecific and doubtful reactions. Considerable

¹² München. med. Wehnschr., 1919, 66, p. 505.

amounts of free hemoglobin did not interfere with the test. Cloudiness of a serum made it difficult to read the reaction.

Salt Solution.—Sodium chloride solution, 0.85%, was made in distilled water. A volumetric flask was used to insure uniformity of concentration. The solution was freshly prepared for each set of tests and was sterilized in the Arnold for 1 hour to reduce bacterial contamination of the tests to a minimum. The salt solution was cooled to room temperature before use.

Serum.—Three tubes, 1 for each antigen and 1 for the serum control, were set up for each test. To each tube was added 0.1 c c of the patient's serum and 1 c c of salt solution. To expedite pipetting, we used 1 c c of salt solution and not 0.9 c c, as indicated in the original test, with no appreciable change in the sharpness of the reactions.

Antigen Dilution.—For each test 0.5 c c of the antigen dilution determined as suitable by the preliminary titration was used. Just before setting up the tests, this antigen dilution was prepared in bulk by calculating the total amount needed for all the tests to be done. The calculated amount of the antigen extract was then placed in a clean, dry, 200 c c Erlenmeyer flask and the proper amount of the alcoholic solution of the cholesterol added. An equal volume of salt solution was then rapidly run into the flask from a buret, and the flask was gently rotated to insure complete mixture of the ingredients. A time interval of 10 minutes was allowed to elapse and then in a similar manner 4 more volumes of salt solution were added and the flask gently rotated as before. This method of dilution insured a uniformity in the antigen concentration. By adding the salt solution too rapidly an antigen dilution of slight opalescence and correspondingly slight sensitiveness was produced. When the solution was added too slowly, the extract dilution was very turbid and was found to be either hypersensitive or subject to spontaneous flocculation.

Serum Control.—To one tube of the set was added 0.5 c c of a solution of absolute alcohol, 1 volume to 5 volumes of saline solution—in other words, an amount of alcohol equivalent to the alcoholic content of the antigen used in the tests. A definite flocculation in this tube would invalidate the test. However, in 1,000 tests we did not obtain such a flocculation in the serum control tube. We found the chief value of the serum control tube to be the elimination, by comparison, of doubtful positives in tests in which there was a considerable amount of bacterial growth.

Antigen Control.—An antigen control was used for each antigen in a series of tests. To 1 c.c. of salt solution was added 0.5 c.c. of the antigen dilution. The control tube should remain unchanged throughout the entire incubation period.

Incubation.—Each tube was shaken individually. All tests were then incubated for 20 hours at 37 C. Provisional readings were made at the end of 20 hours, after which the incubation was continued for 20 hours in a cold room at 14 to 16 C., or in the icebox.

Readings.—A test which showed complete precipitation, or with occasional flocculi in suspension and no opalescence in the supernatant liquid, was rated “++++.”

Tests which showed considerable sedimentation with many fine flocculi in suspension were read as “+++.”

Tests which showed a moderate amount of sedimentation with the supernatant liquid filled with very fine flocculi were read as “++.”

TABLE 1
GROSS COMPARISON OF RESULTS WITH THE WASSERMANN AND THE SACHS-GEORGI TESTS

Agreement		Disagreement	
Negative tests.....	676	Wassermann positive.....	} 45
		Sachs-Georgi negative.....	
Positive tests.....	268	Wassermann negative.....	} 7
		Sachs-Georgi positive.....	
Total tests.....	1000		
Agreement.....	94.4%	Wassermann anticomplementary.....	} 4
		Sachs-Georgi, definite results.....	

Tests which showed a slight fine sediment with a turbid supernatant liquid were rated as “+.” Tests in this class frequently must be centrifugalized at slow speed for a short time to determine whether they are positive or not. On shaking a centrifugalized test, if rated as a “+,” a few well organized white flocculi will be suspended. A centrifugalized test containing considerable bacterial growth will contain a sediment which is gray and finely granular, but which can be entirely dispersed in the supernatant liquid on shaking the tube. All of our tests were read without difficulty with the naked eye.

The Wassermann Technic.—The Wassermann tests which were run in parallel with the Sachs-Georgi tests were performed by the serologist, Miss Rockstraw, in the laboratory of Dr. J. G. Hopkins. Two alcoholic

antigens, and one cholesterol antigen containing 0.2% of cholesterol were used in each test. The alcoholic antigen tests were incubated for 4 hours in the icebox prior to the addition of the hemolytic system. The cholesterolized antigen tests were incubated for one hour in the water bath at $37\frac{1}{2}$ C. before the addition of the hemolytic system. The one-tenth Wassermann is used entirely in this laboratory.

Very early in our work we observed minor variations in the results obtained by the Wassermann and the Sachs-Georgi tests. These variations were most prominent in the weak positive serums, and occurred most frequently in old cases and in patients receiving antisyphilitic treatment. An effort was then made to obtain information on the number of treated cases and to ascertain whether a relationship really existed between treatment of the patient and irregularities of findings by the Wassermann and Sachs-Georgi tests. Likewise, considerable variation existed between the cholesterol and alcoholic antigens in the Wassermann tests, which raised the question as to which of these antigens the Sachs-Georgi results most nearly paralleled. To show these findings in a brief and comprehensive manner, the following tables were devised:

TABLE 2
COMPARISON OF THE WASSERMANN AND SACHS-GEORGI TESTS IN DETAIL

Number	Wassermann Tests		Detailed Comparative Readings of the S.-G. Tests					Percentage Agreement as to Positive or Negative Nature of Result
	Cholesterol Antigen	Alcoholic Antigen	++++	+++	++	+	—	
126	++++	++++	92	24	4	3	3	97.6
10	Cholesterol > Alcoholic							
21	++++ or +++	++++ or ++	5	2	1	1	1	90.0
58	++++ or +++	+ or —	5	3	7	5	1	96.2
27	Cholesterol < Alcoholic							
12	+++ or ++	++++ or +++	10	16	16	13	3	94.8
5	+ or —	++++ or +++	1	0	3	14	9	59.3
16	+++	+++	2	3	3	3	1	91.7
33	++	++	0	0	4	1	0	100.0
683	Cholesterol > Alcoholic							
4	++ or +	+ or —	0	1	1	12	2	87.5
	Cholesterol < Alcoholic							
	+ or —	++ or +	0	0	3	7	23	30.3
	—	—	0	0	0	3	2	60.0
	(Anticomplementary)		0	0	2	5	676	98.9
			2	0	0	0	2	—
1000		117	49	44	67	723	

It should be noted that in the 4 cases which were anticomplementary to the Wassermann test a definite diagnosis was obtained by the Sachs-Georgi test in each case. In order to determine the reliability of the Sachs-Georgi findings in these cases the histories of the cases were

obtained. One of the cases which was anticomplementary to the Wassermann and negative to the Sachs-Georgi test was a treated case which had evidently "cleared up" under treatment. Another case which was likewise negative in the Sachs-Georgi test and anticomplementary in the Wassermann test presented an absolutely negative syphilitic history. Of the 2 cases which were anticomplementary in the Wassermann tests and + + + + in the Sachs-Georgi tests, one was that of a tertiary syphilitic. In a subsequent Wassermann test 0.01 c c of this patient's serum in a 1:5 dilution was + + + +. The other was a new case in the gynecologic department of the Vanderbilt Clinic in which the patient showed, on examination, venereal warts of the vagina.

TABLE 3
COMPARISON OF THE WASSERMANN AND SACHS-GEORGI TESTS IN TREATED CASES

Number of Treated Cases	Wassermann Tests		Detailed Comparative Readings of the S.-G. Tests					Percentage Agreement as to Positive or Negative Nature of Result
	Cholesterol Antigen	Alcoholic Antigen	++++	+++	++	+	—	
28	++++ Cholesterol > Alcoholic	++++	16	7	3	1	1	96.4
4	++++ or +++	+++ or ++	2	0	1	0	1	75.0
12	++++ or +++ Cholesterol < Alcoholic	+ or —	3	1	4	4	0	100.0
28	+++ or ++	++++ or +++	5	6	8	7	2	92.8
16	+ or —	++++ or +++	1	0	1	7	7	56.2
8	+++	+++	1	3	2	1	1	87.5
4	++ Cholesterol > Alcoholic	++	0	0	3	1	0	100.0
11	++ or + Cholesterol < Alcoholic	+ or —	0	0	0	10	1	90.9
21	+ or —	++ or +	0	0	2	4	15	28.6
3	+	+	0	0	0	1	2	33.3
69	—	—	0	0	2	3	64	92.7
204	28	17	26	39	94	

Of the 204 treated cases, 84 would have been rated + + and above with the cholesterol antigen, 11 would have been + to + +, and 109 + or —. Of the same number of cases the alcoholic antigen would have rated 88 + + and above, 21 + to + +, and 95 + or —. The Sachs-Georgi test would rate 71 of the same number of treated cases + + and above, and 133 + or —.

An analysis of table 3 reveals the following facts:

(1) The Sachs-Georgi test was more sensitive than the Wassermann test in 17 treated cases. Of these cases, 12 or 70.5% were strong positives by both tests.

(2) The Wassermann reaction was more sensitive than the Sachs-Georgi test in 98 treated cases. Sixty-one of these cases, or 62.2%,

were strong positives to one or both Wassermann antigens. These figures would indicate that the Wassermann test is more sensitive than the Sachs-Georgi test in weak positive cases under treatment.

(3) The two tests were equally sensitive in 87 treated cases. Of these tests of equal sensitiveness to both methods of diagnosis, 16 were + + + +, 7 were weak positives, and 64 were negative.

In a comparison of the percentage of agreement in the two tests in all cases and in treated cases, all groups of tests, as presented in tables 2 and 3, compared favorably except in the Wassermann group in which cholesterol antigen gave a + + + + or + + + reaction, and the alcoholic antigen a + + + or + +, the agreement being 90% for all cases in the group and 75% for the treated cases in the same group. Also, in the + Wassermann group to both antigens, the percentage of agreement is 60 for all cases and 33.3 for treated cases in the same group. With only 3 exceptions, the percentages of agreement for all cases in the various groups are from 1 to 4 points higher than the corresponding percentages of the treated cases. Of the 3 exceptions, the group in which the Wassermann reaction is + + to both antigens, the agreement is 100% in both tables. In the remaining 2 groups the percentages for all cases are 3 or 4 points lower than the corresponding percentages for treated cases. From a comparison such as the one just presented, it is concluded that the Sachs-Georgi test is not as sensitive as the Wassermann test in cases of patients undergoing antisyphilitic treatment. This idea is supported by the fact that 30 patients under treatment cleared up sooner to the Sachs-Georgi test than they did to the Wassermann test, whereas only 5 treated patients gave a positive Sachs-Georgi reaction when the Wassermann test was negative.

Referring again to table 2, it will be noted that in the strong positive groups in which the cholesterol and alcoholic antigen of the Wassermann did not agree, the Sachs-Georgi results agreed more closely with the cholesterolized Wassermann antigen than with the alcoholic antigen, there being 73 cases in which the Sachs-Georgi test paralleled closely the results obtained by the cholesterol Wassermann antigen, and 43 cases in which the reverse was true. The question could be raised as to why the cholesterol "fortified" antigen should give a weaker reaction than the alcoholic antigen. (For it will be noted in table 2 that in 85 cases the alcoholic antigens in the Wassermann test gave a stronger reaction than the cholesterol antigen, while 31 cases gave reactions in the reverse order.) It is well known that a cholesterol antigen is

supersensitive when subjected to the icebox treatment. Cholesterol being one of the higher alcohols ($C_{26}H_{43}OH$) and fat-like in character, it is possible that in the icebox treatment, cholesterol aggregates are formed which are of such a size and nature as to be able to fix complement mechanically in a manner comparable to the ultramicroscopic precipitate in the positive Wassermann test. Cholesterol is found in appreciable quantities in the bile, and a patient who is troubled with icterus would have some cholesterol in the blood serum which when subjected to the icebox treatment would act in a manner similar to a cholesterol "fortified" antigen and thus give a degree of complement fixation which would seem to indicate that an alcoholic antigen is more sensitive than a cholesterol antigen.

Several cases were encountered in this study which resulted in a total disagreement between the Wassermann and Sachs-Georgi findings. In order to determine which method of diagnosis was the more reliable, histories of the cases were obtained. Since no general classification is possible, these cases are presented in brief form in tables 4 and 5.

In addition to the cases of total disagreement listed, there were 28 cases of partial disagreement, such as a negative result with the cholesterol Wassermann antigen and a +, ++ or +++ results with the alcoholic antigens, in which the parallel Sachs-Georgi tests were negative. Twenty-one of these patients gave a positive syphilitic history or were under treatment at the time of the tests. The remaining 7 patients presented doubtful histories. One case, in particular, gave a history of severe headaches. A lesion was denied by the patient. A Wassermann test made on April 4 was: cholesterol antigen negative, alcoholic antigens + + + +, Sachs-Georgi test negative. Subsequent Wassermann tests made on April 7 and 11 were, respectively, cholesterol antigen negative, alcoholic antigens + +, and negative for all antigens. Another case of an opposite character likewise gave no history of an initial lesion. The patient had been treated for 4 years for tuberculosis of the throat. At the time of the test the patient complained of rheumatic pains and had on the chin a serpiginous crypt irregular in shape with a scaly crusted center. The Wassermann test was negative with the cholesterol antigen and + with the alcoholic antigen. The parallel Sachs-Georgi test was negative. A subsequent Wassermann test was + + + +.

Three cases were encountered which gave a ++ or +++ reaction with the cholesterol Wassermann antigen and negative results with the alcoholic antigens, while the parallel Sachs-Georgi tests were nega-

tive. Two of these patents gave positive syphilitic histories. The remaining patient gave no definite history of syphilis. The serum of this patient was unfit for any test, being very turbid and contained a great amount of released hemoglobin. In the entire group in which the Wassermann test was positive in the cholesterol antigen tests and

TABLE 4
CASES OF DISAGREEMENT BETWEEN THE WASSERMANN AND SACHS-GEORGI TESTS
(SACHS-GEORGI NEGATIVE)

Patient	Wassermann			Sachs-Georgi	History
	Cholesterol Antigen	Alcoholic Antigen	Alcoholic Antigen		
J. V.	++++	++++	++++	—	Lesion denied. Paralysis of left recurrent laryngeal nerve, partial paralysis of right recurrent laryngeal nerve. One knee jerk absent. Under antisyphilitic treatment
T. S.	++++	++++	++++	—	Contracted syphilis 9 years ago. Just prior to present test developed osteomyelitis as a result of an injury in an automobile accident
A. E.	++++	++++	++++	—	Developed a chancre 7 years ago. Received 2 antisyphilitic treatments at that time. At present complains of headache and malaise. Pupils sluggish
Harris. (Baby of V.)	++	++++	++++	—	Cord Wassermann. Mother was receiving antisyphilitic treatment at the time of the present tests
M. G.	+	++++	++++	—	Tertiary syphilis, under treatment at the time of the test
B. B.	+	++++	++++	—	Children were positive to the Wassermann test. Received antisyphilitic treatment prior to test
S. C.	+	++++	++++	—	A new case in the Vanderbilt Clinic; migraine. Symptoms began 2 years ago with gradual onset. Clinical diagnosis of syphilis doubtful
M. A.	+++	+++	+++	—	Under antisyphilitic treatment at time of the test
S. C.	++	+++	+++	—	A treated case. Treatment was discontinued 2 months prior to test
V. B.	+++	++	++	—	Previously diagnosed as syphilis. Under treatment at time of the test
M. S.	+	+	++	—	Gonorrheal infection 10 years ago. Complains of pain in the feet. Diagnosed subcutaneous syphiloma. A subsequent Wassermann test was ++++ for all antigens

* Most of the cases in this group represent old cases, congenital syphilis and treated cases which "cleared up" early to the Sachs-Georgi test. The Sachs-Georgi test appears to be unreliable when used with cases of this nature.

negative to the alcoholic antigens, the Sachs-Georgi tests were positive in 18 cases and negative in 3 cases. Of the 18 cases of agreement of the Wassermann cholesterol antigen and the Sachs-Georgi test, 11 were treated cases and 3 cases presented positive syphilitic histories.

In the two groups just discussed, composed of weak positive (not agreeing in the results obtained with the cholesterol and the alcoholic

antigens) according to the Wassermann test, the Sachs-Georgi tests gave results which agreed exactly with the results obtained with the cholesterol Wassermann antigen in 46 of the 49 cases tested.

SUMMARY

When roughly classified into "positive" and "negative," the Sachs-Georgi tests agreed with the Wassermann tests in 94.4% of 1,000 cases tested.

The Sachs-Georgi test appears to be less sensitive than the Wassermann reaction in patients receiving antisyphilitic treatment and in cases of cerebrospinal syphilis.

The Sachs-Georgi test is just as sensitive as the Wassermann test in untreated cases of primary, secondary and tertiary syphilis.

TABLE 5
CASES OF DISAGREEMENT BETWEEN THE WASSERMANN AND SACHS-GEORGI TESTS
(SACHS-GEORGI POSITIVE)

Patient	Wassermann (All Antigens)	Sachs-Georgi	History
A. T.	—	++	A treated case. Treatment was discontinued 6 weeks prior to test
M. P.	—	++	Receiving antisyphilitic treatment at time of the test
F. S.	—	+	Antisyphilitic treatment discontinued 3 weeks
T. McG.	—	+	Chancere on the penis 20 years ago. Chronic eczema on left shin. Tertiary syphilis
C. M.	—	+	Fibroid (no record of any kind could be obtained of this case)
L. S.	—	+	Tertiary syphilis; under treatment at time of test
A. W.	—	+	Antisyphilitic treatments were discontinued 4 months prior to test

All of these cases, except T. McG. and C. M., may be interpreted as treated cases which "cleared up" to the Wassermann test earlier than to the Sachs-Georgi.

The specificity of the Wassermann alcoholic antigen under the four-hour icebox treatment can be questioned when the presence of bile salts in the patient's serum is ignored. With this unknown quantity controlled, the Sachs-Georgi test would show a still more favorable comparison with the Wassermann reaction as a routine test.

In four cases in which the patient's serum was anticomplementary in the Wassermann test, the Sachs-Georgi test gave a definite and reliable diagnosis in each case.

Because of the simplicity and reliability of the test, as shown by the data herein presented, the Sachs-Georgi test should attain a range of usefulness in the diagnosis of syphilis far in excess of that accredited to it at the present time.